

VOLTAGE CLAMP ELECTROPHYSIOLOGY
SCREENING OF SELECTED COMPOUNDS FROM
Zizyphus mauritiana AND *Myristica fragrans* ON
SPECIFIC GABA RECEPTORS

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**VOLTAGE CLAMP ELECTROPHYSIOLOGY SCREENING OF SELECTED
COMPOUNDS FROM *Zizyphus mauritiana* AND *Myristica fragrans* ON SPECIFIC
GABA RECEPTORS**

By

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This thesis is dedicated to my best friends;

Fuad Ikhwan, Harizal Senik and Norzihan Ab Rahman

"True friends never walk away"

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LIST OF ABBREVIATIONS

4-PIOL	5-(4-piperidyl)-3-isoxazolol
5-HT	serotonin receptors
μE	microelectrode
μg	microgram
μL	microliter
μM	micromolar
μm	micrometer
A	ampere
AMPA	2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid
ATP	adenosine triphosphate
bp	base pair
C	carbon
Ca	calcium
CaCl ₂	calcium chloride
CC	column chromatography
CHCl ₃	chloroform
cm	centimeter
CNS	central nervous system
CTP	cytidine triphosphates
Da	dalton
DCM	dichloromethane
DEPC	diethylpyrocarbonate
DH5-α	strain name for <i>E. coli</i>

DMCM	methyl-6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
DTT	dithiothreitol
<i>E.coli</i>	<i>Escherichia coli</i>
EC ₅₋₁₀	GABA concentration eliciting between 5-10% of the maximal current
EC ₅₀	concentration that increase the amplitude of GABA current by 50%
EGCG	epigallocatechin gallate
EtOH	ethanol
g	gram
GABA	gamma-amino butyric acid
GCMS	gas chromatography mass spectrophotometer
GTP	guanosine triphosphate
h	hour
HCl	hydrochloric acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HIAA	hydroxyindoleacetic acid
HPLC	high-performance liquid chromatography
<i>I</i>	current
<i>I_m</i>	injected current

KCl	potassium chloride
kg	kilogram
KHz	kilohertz
LB	lysogeny broth
LGIC	ligand-gated ion channel
LHA	liquid-handling arm
M	molar
mbar	milibar
MCE	methanol crude extract
ME	microelectrode
MeOH	methanol
mg	milligram
MgCl ₂	magnesium chloride
min	minute
mL	mililiter
mm	millimeter
mM	milimolar
MMDA	3-methoxy-4, 5- methylene-dioxyamphetamine
mV	milivolt
N	nitrogen
NaCl	sodium chloride
NaOH	sodium hydroxide
ND 96	buffer solution for oocyte recording
n_H	Hill coefficient

n	nano
NH ₃	ammonia
nm	nanometer
NMDA	<i>N</i> -Methyl-D-aspartic acid
OD	the density of the cells value
OR 2	oocyte ringer number 2
P	phosphate
RE	restriction enzyme
RNA	ribonucleic acid
rpm	rotation per minute
s	second
SOC	super optimal broth
TBPS	tbutylbicyclophosphorothionate
TBE	tris/borate/EDTA
TLC	thin layer chromatography
THIP	4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol
TMS	tricaine methanesulfonate
T	transmembrane domain
UTP	uridine-5'-triphosphate
UV	ultraviolet
V	volume
V _c	command voltage
V _m	membrane voltage
volt	voltage

LIST OF SYMBOL

%	percent
α	alpha
β	beta
γ	gamma
δ	delta
ε	epsilon
θ	theta
ρ	rho
Ba^{2+}	barium
Ca^{2+}	calcium
Cd^{2+}	cadmium
Cl^-	chloride
K^+	potassium
La^{3+}	lanthanum
Mn^{2+}	manganese
Mg^{2+}	magnesium
Na^+	sodium
Sr^{2+}	strontium
Zn^{2+}	zinc
$^{\circ}\text{C}$	degree Celsius

LIST OF PRESENTATIONS AND PUBLICATIONS

Tarmizi, C.H., Baburin, I., Hering, S., Osman, H., Mohamad, H., Abdullah, J. The inhibitory effect of the nutmeg essential oil on GABA (A) receptors comprising of $\alpha_1\beta_2\gamma_{2s}$ subunits. *Planta Medica Journal* (Forthcoming).

Islam, M.R., Muthuraju, S., **Tarmizi, C.H.**, Zulkifli, M.M., Osman, H., Mohamad, H., Abdullah, J. Anticonvulsant activity of α -terpineol isolated from *Myristica fragrans*. *ASM Journal* (Forthcoming).

Tarmizi, C.H., Hering, S., Baburin, I. Pharmacological properties of GABA (A) receptors by the essential oil of *Myristica fragrans* using the electrophysiological technique on *Xenopus* sp. oocyte. National Conference of Medical Sciences, Universiti Sains Malaysia, 22-23 May 2008. pp 37.

**APITAN VOLTAN SECARA ELEKTROFISIOLOGI TERHADAP
PENYARINGAN SEBATIAN TERPILIH DARIPADA *Zizyphus mauritiana*
DAN *Myristica fragrans* PADA RESEPTOR GABA SPESIFIK**

ABSTRAK

Kajian ini memfokuskan pada kesan ekstrak metanol daripada daun *Zizyphus mauritiana* dan minyak pati daripada isirung *Myristica fragrans* pada fungsi reseptor GABA (A). Kedua-dua ekstrak daun *Zizyphus mauritiana* dan minyak pati isirung *Myristica fragrans* diuji terhadap oosit *Xenopus laevis* yang mengandungi reseptor GABA (A) jenis $\alpha_1\beta_2\gamma_{2s}$. Sistem perfusi automatik pantas yang menggunakan dua mikroelektrod pada kepekatan GABA EC₅₋₁₀ telah digunapakai di dalam kajian ini. GABA atau asid aminobutirik-gamma adalah salah satu neurotransmitter yang paling banyak dipelajari dan merupakan neurotransmitter perencatan utama dalam sistem saraf pusat mamalia dan terlibat dalam keseimbangan di dalam aktiviti pengaktifan dan perencatan sel-sel neuron. GABA menjalankan tindakan perencatan melalui pengaktifan pada reseptor GABA. Reseptor GABA ini boleh dibahagikan kepada reseptor GABA (A), (B) dan (C). Reseptor GABA (A) adalah suatu reseptor perencatan yang terdiri daripada kompleks protein transmembran pentamer yang membentuk saluran anion intrinsik yang telap kepada ion Cl⁻ yang terletak di tengah kompleks. Saluran ion ini kekal dalam keadaan tertutup dan boleh dibuka dengan pengikatan GABA pada kompleks yang turut dimodulasi oleh pelbagai sebatian yang bertindak di tapak pengikatannya. Pembangunan kajian ke atas ligan yang berkemampuan untuk mengikat pada tapak pengikatan reseptor GABA (A) menjadi keutamaan dalam kajian terapeutik. Sehingga kini terdapat sebatian tumbuhan yang

dikenal pasti mempengaruhi fungsi ionotropik reseptor GABA (A). Ujian ke atas 100 $\mu\text{L/mL}$ minyak pati menunjukkan peningkatan arus GABA sekitar 100%, namun ekstrak daun *Zizyphus mauritiana* pada kepekatan 100 $\mu\text{g/mL}$ tidak menunjukkan perubahan yang signifikan secara statistik terhadap I_{GABA} . Dalam ujian kedua, tiga sebatian sintetik yang terdapat dalam minyak pati isirung *Myristica fragrans*; limonene, α -terpineol dan myristicin serta beberapa bahagian ekstrak daripada daun *Zizyphus mauritiana* telah diuji terhadap reseptor GABA (A) pada kepekatan GABA 5-10% daripada tindak balas maksima. α -Terpineol adalah sebatian paling berpotensi dan paling efisien kerana menunjukkan peningkatan tertinggi pada I_{GABA} iaitu sehingga $229.6 \pm 23.8\%$ dan modulasi maksimum $326.3 \pm 43.8\%$ pada 500 μM dengan nilai EC_{50} sekitar 88 μM . Myristicin juga menunjukkan potensi yang signifikan dari segi statistik iaitu pada 500 μM dengan peningkatan $237.6 \pm 35.1\%$ pada I_{GABA} .

**VOLTAGE CLAMP ELECTROPHYSIOLOGY SCREENING OF
SELECTED COMPOUNDS FROM *Zizyphus mauritiana* AND *Myristica
fragrans* ON SPECIFIC GABA RECEPTORS**

ABSTRACT

This study focused on the effects of the methanol crude extract of *Zizyphus mauritiana* leaves and the essential oil of the mesocarp of *Myristica fragrans* on the function of GABA (A) receptors. Both crude and essential oil were tested on the *Xenopus laevis* oocytes that comprised of GABA (A) receptors $\alpha_1\beta_2\gamma_{2s}$ subtype. An automated fast perfusion system that applied the two microelectrodes voltage clamp measurement technique at EC₅₋₁₀ of GABA was used. GABA or gamma-aminobutyric acid is the major inhibitory neurotransmitter in the mammalian central nervous system and involves in the balance between excitation and inhibition in the neuronal cells. GABA expresses the inhibition actions by activation of the GABA receptors. These receptors can be divided into GABA (A), GABA (B) and GABA (C) receptors. GABA (A) receptors are pentameric protein inhibitory receptors that are arranged in a circle to form an intrinsic anion channel permeable to Cl⁻ ions in the middle. These ion channels remain close until opened by specific agonist, GABA and modulated by a variety of compounds that act at its binding sites. The development of new ligands that which has the ability to bind on the GABA (A) receptors binding sites are the primary targets in current therapeutic study. There are many natural substances that have been identified to show potential in GABA (A) receptors activation thus influencing the functions of these ionotropic GABA (A) receptors. When tested at 100 μ L/mL the essential oil enhanced *I* GABA by about

100%, however, the crude of *Zizyphus mauritiana* leaves at 100 µg/mL showed no statistical significance effect of *I* GABA. In the second assay, three synthetic compounds found in the essential oil; limonene, α-terpineol and myristicin and the fractions of *Zizyphus mauritiana* leaves extract were tested on GABA (A) receptors at GABA concentration eliciting 5-10% of the maximal response. α-Terpineol was the most potent and most efficient compound because it modulated the highest enhancement of *I* GABA by $229.6 \pm 23.8\%$ and the maximum current stimulation was $326.3 \pm 43.8\%$ at 500 µM with the EC_{50} value ≈ 88 µM. Myristicin also exhibited statistical significance of potentiation at 500 µM with $237.6 \pm 35.1\%$ enhancement of *I* GABA.

CHAPTER 1

INTRODUCTION

1.1 *Zizyphus mauritiana* Lam.

Zizyphus mauritiana Lam. or commonly known as Indian jujube, is a genus of *Zizyphus* and under the family of Rhamnaceae. This species is an evergreen plant that can reach up to 40 feet or more in height (Figure 1.1). *Zizyphus mauritiana* is originally from India but now can be found widely especially in dry and rocky places. This plant is hermaphrodite (it has both male and female organs) and is pollinated by insects. The leaves are almost round-shaped and dark green in colour. The flowers are yellowish-white, borne in clusters along the leaf axils and this is shortly followed by the rounded fruit. The mature fruit is usually light yellowish-orange to almost brown and 1-1.5 inch in diameter which tastes like green apple. If the fruit is too mature it tastes almost like dates.